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ON THE PIGMENTATION OF URIC ACID CRYSTALS DEPOSITED FROM URINE.

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(Plate III.)

There is no more familiar fact of clinical chemistry than the coloration of uric acid crystals deposited from urine, but the nature of the pigment or pigments to which the crystals of this colourless acid owe their tints has never, as far as I am aware, formed the subject of a systematic study.

It is true that Wetzlar,[1] Duvernoy,[2] and others, extracted the colouring matter from such sediments by means of boiling water or alcohol, and examined some of its properties, but, at the time they did so, next to nothing was known about the urinary pigments, and consequently their results threw but little light upon the question. Künkel[3] has detected the presence of iron in sediments of uric acid thrown down by the addition of hydrochloric acid to urine, and regards it as a constituent of the included colouring matter; but, although the correctness of the observation cannot be questioned, there are, as I shall show, reasons for doubting the interpretation put upon it. Most modern authors who refer to the subject offer no suggestion at all as to the nature of the contained pigment, confining themselves to a description of the appearance of the crystals; but in a few works we meet with the statement that the sediments owe their colour to uroerythrin, the pigment of pink urates.

From the point of view of their pigmentation, deposits of uric acid may be conveniently classified into four groups, as follows:—

1. "Cayenne pepper" deposits, which are the commonest, and which, when seen in bulk, have a more or less intense red colour. They are composed of crystals, which, examined in detail, have a warm orange tint, occasionally verging upon red.

2. Yellow or fawn coloured deposits, consisting of yellow crystals which often show a tinge of brown.

[1] "Beiträge zur Kentniss des menschlichen Harns," etc. Frankfort-on-the-Main, 1821.

[2] "Chemisch-medicinische Untersuchungen über den menschlichen Urin." Stuttgart, 1835.

[3] *Sitzungsb. d. phys.-med. Gesellsch. zu Würzb.* 1881, p. 69.

3. Deposits coloured by abnormal pigments occasionally present in urine, which appear brown or black when seen in bulk.

4. Brown deposits thrown down by the addition of mineral acids to urine.

It is obvious that no single colouring matter can produce all the tints observed even in the spontaneously deposited crystals, and it is hardly possible to doubt that they owe their ground tint to the substance, whatever be its nature, which gives the normal urine its yellow colour.

The influence of the contained pigments is not confined to the mere tinting of the crystals, for they also appear to play a very important part in determining their form. Duvernoy clearly recognised this fact, which has been more recently studied by Ord [1] as an example of the influence of colloids upon crystalline form. After repeatedly redissolving the urinary crystals in water, Dr. Ord ultimately obtained specimens which were colourless, and had the tabular forms of crystals of pure uric acid.

Doubtless there are other factors simultaneously at work in moulding the crystals into the almost innumerable shapes which they assume; and of these factors the degree of acidity of the liquid is, as Sansom [2] has shown, one of the most potent.

Sir William Roberts [3] attributes to the pigments yet another important action, namely, that of retarding the separation of free uric acid from solutions of the quadrurates.

The line of investigation which throws most light upon the question of coloration is the precipitation of crystals of uric acid from solutions containing the several urinary pigments in as pure condition as possible, and I propose to speak first of the results of a series of experiments of this kind.

The solutions of urate employed were obtained from dry and clean snake's urine, which, when treated with hot water, yields, as Sir William Roberts has shown, a solution of quadrurates from which colourless crystals of uric acid are in a short time deposited. As a rule, this material was treated with a hot aqueous solution of neutral sodium phosphate, and to the solution so obtained, after it had been freely diluted with water, a small quantity of acid sodium phosphate was added. When rapidly deposited from such solutions the colourless crystals of uric acid have the form of very thin rectangular plates, but when more slowly formed they appear as more massive square or oblong tables, or as derivatives of such forms. (Plate III. Fig. 1.)

Numerous experiments were made with uroerythrin, which was obtained in a state of tolerable purity, but not entirely free from yellow pigment, by a method described by Riva,[4] which consists in

[1] "The Influence of Colloids upon Crystalline Form and Cohesion," 1879, p. 52.

[2] Quoted by Beale, "Kidney Diseases and Urinary Deposits," 3rd edition, 1869, p. 371.

[3] "Croonian Lectures on Uric Acid, Gravel, and Gout," 1892, p. 46.

[4] *Gazz. med. di Torino*, 1892, vol. xliii. p. 3.

washing pink urate sediments with iced water, and afterwards with cold alcohol; solution of the washed sediment in warm water; shaking the solution so obtained with pure amylic alcohol, which extracts the uroerythrin with great avidity; evaporation of the amylic solution, and solution of the residue in distilled water.

Such solutions of uroerythrin are, as Riva and Zoja have shown, rapidly decolorised by light, and must therefore be kept in a dark place, or must at least be protected from actinic light.

Uric acid crystals, thrown down from solutions of uroerythrin, have a delicate pink colour, which is quite unlike that of the natural urinary crystals, and is more absolutely pink the purer the material employed. The prevailing shape recalls that of the "razor shell" of our coasts, and the individual crystals are apt to be grouped together into rosettes, or to assume a cruciform arrangement. (Plate III. Fig 2.)

Similar pink crystals may be obtained in a simpler way by allowing the aqueous solution of a deeply-coloured pink urate sediment, which has been thoroughly washed both with water and with alcohol, to stand in a dark place. Rosettes and clusters of prismatic crystals are in time deposited, together with a few boat-shaped ones, which have a yellow tint; for urate sediments always contain some yellow pigment, which is not removed by washing.

It is evident from the above results that uroerythrin has a great affinity for uric acid, and when present in the urine must have a share in the coloration of the crystals deposited therefrom; but it is equally evident that uroerythrin is not the only colouring matter of the urinary sediments. The presence of some uroerythrin in them is the rule rather than the exception, for, as Riva and Zoja have shown, by shaking the urine with amylic alcohol the presence of this pigment may very frequently be demonstrated even in pale urine; and it is well known that the urine of healthy individuals not infrequently deposits pink urates.

A further series of experiments showed that urobilin, which is by some regarded as the chief colouring matter of normal urine, is not one of the pigments concerned in the coloration of the deposits; for it was repeatedly found that uric acid crystals, thrown down from solutions of urobilin, were hardly appreciably tinted when examined in bulk, and under the microscope appeared practically colourless. Moreover, and this is a point of considerable importance, the crystals assumed forms identical with those met with in specimens deposited from pure aqueous solutions of urate.

Hæmatoporphyrin, extracted from urine, proved to be equally devoid of the power either of colouring or of modifying the form of uric acid crystals.

Of the pre-formed urinary pigments there remains to be considered the yellow colouring matter of normal urine, to which Thudichum has assigned the name of Urochrome. Of the existence of such a pigment,

as distinct from urobilin, I am convinced, and in a paper recently communicated to the Royal Society[1] I described in detail a method by which it can be isolated, in a condition of approximate purity, which differs from those previously employed in dispensing with the use both of mineral acids and of metallic precipitants.

Urate sediments always contain some of this pigment, and from the palest urate sediments, which show no pink tint, aqueous solutions may be obtained which deposit "whetstone" crystals of a pale yellow colour. In some cases there are found in the urine—together with such uroerythrin—free urate sediments, crystals of uric acid which have a yellow or brownish-yellow colour when examined under the microscope, and which appear yellow or fawn coloured when seen in bulk. There is, therefore, good reason for supposing that such sediments, which constitute the second group in the classification given above, do not owe any of their colour to uroerythrin.

Numerous experiments were made by dissolving pure colourless urate in solutions of the yellow pigment, prepared by the method above referred to, and it was found that this substance had a very great affinity for uric acid. It was, however, much more difficult to obtain satisfactory specimens than when solutions of uroerythrin were employed.

The uric acid was apt to be deposited very rapidly in the form of an almost impalpable powder, which consisted of very minute and faintly tinted crystals, but these showed a marked tendency to assume the whetstone form. Aqueous solutions of the isolated yellow pigment are very prone to undergo decomposition, which is evidenced by a change of tint from yellow to brown, and this tendency, which is especially pronounced when the liquid is rendered acid, introduces a further difficulty, and when larger and more slowly formed crystals were obtained they usually showed a brownish tint (Plate III. Fig. 3). The most satisfactory specimens bore a very close resemblance, both in colour and form, to the paler variety of urinary sediments; and there cannot, I think, be any doubt that it is this yellow pigment that supplies the ground tint of the natural crystals, and plays a conspicuous part in moulding them to the whetstone shape, or to the more complex forms produced by the agglomeration of the boat-shaped crystals.

Since the yellow pigment yields no spectroscopic absorption bands, and gives no characteristic reactions by which it can be recognised with certainty in such minute quantities as are here available, there is little hope of demonstrating its presence in the crystals by other methods; but it can easily be shown that uroerythrin exists in "cayenne pepper" sand in the following way:—

The washed sediment is boiled in water or alcohol until its colour is to some extent removed, and the coloured extract is evaporated to dryness.

[1] *Proc. Roy. Soc. London*, 1894, vol. lv. p. 394.

The solid pigmentary residue so obtained is then touched with a glass rod, which has been dipped in a solution of sodium or potassium hydrate, when a green colour is developed at the point of contact.

This property of yielding a green colour with alkalies is peculiar to uroerythrin among the urinary pigments, and by means of this test the presence of uroerythrin may be demonstrated in crystals which have only a light red colour in bulk, and individually have a bright orange tint.

Moreover, such crystals may be found embedded in pink urate sediments, and sometime exhibit a distinct pink tint near their edges.

It would seem, then, that the pigments concerned in the coloration of cayenne pepper sand are the yellow pigment and uroerythrin, and by the admixture of these two substances, in varying relative proportions, the majority of the tints observed in the naturally deposited urinary crystals may be explained. On the other hand, those sediments which lack the usual red colour, when seen in bulk, do not contain uroerythrin, but may be supposed to owe their tint to the yellow pigment alone.

This interpretation of the observed phenomena affords no explanation of the presence of iron in the sediments. Like Künkel I have detected traces of this metal in specimens thrown down by hydrochloric acid, as well as in the natural sediments; indeed, the natural deposits yield, as a rule, a much more conspicuous iron reaction than do those precipitated by acid, and when burnt on a platinum dish sometimes leave an appreciable amount of reddish ash. For example, whereas 0·09 grm. of sediment thrown down from normal urine by means of hydrochloric acid gave, after combustion, only a very feeble colour reaction with potassium sulphocyanide, such much smaller quantities of natural sediments as weighed only 0·0152 and 0·0293 grm. yielded, when similarly treated, a very pronounced colour.

It should be mentioned that the amount of iron varies much in different specimens, and bears no obvious relation to the depth of their coloration.

It can be definitely stated that the iron contained in the crystals is not contained in the yellow pigment or in the uroerythrin, and if, as Künkel suggests, it is a constituent of the included colouring matter, it must be concluded that some other pigment than those mentioned has a share in the coloration of the crystals, and one very rich in iron, seeing that the sum of the included pigments constitutes but a very small fraction of the total mass of the sediments. Of the presence of such an iron-containing pigment there is no evidence other than this, but the point requires further investigation, since it promises to throw light upon the nature of the unknown compound that contains the small amount of iron, which is constantly met with in urine.

In the yellow pigment, as obtained by my process, I have, indeed, detected extremely minute traces of iron, almost certainly of the nature

of an impurity, for no appreciable reaction with sulphocyanide was obtained after the combustion of specimens of the isolated pigment exceeding in weight the specimens of natural uric acid sediment dealt with in the experiments referred to above.

Specimens of uroerythrin gave, after combustion, no sulphocyanide reaction, and although the quantities employed were small, and did not exceed 4 or 5 mgrms., it may, I think, be confidently stated that uroerythrin is also an iron-free pigment.

Among the abnormal pigments which modify the colour of the sediments of the third group, those of the bile occupy a prominent place.

As the late Professor Ultzmann of Vienna used to point out, sediments of uric acid, deposited in urine containing bile pigments, have a brown instead of a red colour. The tint varies considerably according to the extent to which the conversion of bilirubin into biliverdin has taken place. In urines rich in bilirubin the deposits have a ruddy brown colour, and the tint of the individual crystals is an unusually rich and warm orange; but when, on the other hand, there is much biliverdin present the colour of the deposits is leathery brown, and the individual crystals show a peculiar greenish tinge (Plate III. Fig. 4). The forms of the crystals are also conspicuously modified, especially by biliverdin, and in some instances urines rich in this pigment deposit rosettes of prismatic crystals, whereas in other specimens which I have examined the deviation from the usual urinary types has been less marked.

Blood pigments have no such effect, and sediments deposited from urines containing blood show no modification of tint.

The dark brown or black products formed by the oxidation of phenol derivatives also possess the power of colouring the crystals to a conspicuous degree. My attention was called by Dr. F. W. Andrewes to the fact that in cases of carboluria uric acid sediments, when present are perfectly black. Examined under the microscope the individual crystals are of so deep a brown colour that they are rendered almost opaque (Plate III. Fig. 6).

Lastly, some at least of the little understood brown or black substances which are formed by the action of mineral acids upon urine have a similar power, as is shown by the reddish-brown colour of the crystals thrown down by means of hydrochloric acid (Plate III. Fig. 5).

It is probable that the brown tint of such specimens is largely due to the products of decomposition of the yellow pigment, such as the uromelanine of Thudichum, but it is also possible that the indigo pigments have a share in its production, for from impure solutions of yellow pigment, containing indoxyl sulphate, I have obtained, on the addition of hydrochloric acid, crystals which were obviously tinted with indigo blue. Dr. Ord,[1] too, has obtained crystals encrusted with and penetrated

[1] *Trans. Path. Soc. London*, 1892, vol. xliii. p. 195.

by indigo blue, by the addition of mineral acid to urines rich in indoxyl sulphate.

On the other hand, the indigo pigments cannot be supposed to have any share in the coloration of the naturally deposited crystals, since these pigment are, as far as I am aware, only produced spontaneously in alkaline urines.

Conclusions.

1. Of the true urinary pigments, which exist ready formed in urine, only the normal yellow pigment (urochrome) and uroerythrin appear to possess the property of colouring uric acid crystals deposited from their solutions.

2. The yellow pigment, being a constant constituent of the urine, always furnishes the ground tint of the crystals, and plays the more important part in determining their form; the whetstone or canoe shape being that which this substance specially tends to produce.

3. In the majority of instances uric acid crystals, which are spontaneously and rapidly deposited from urine, contain uroerythrin also, and it is to this pigment that the sediments owe their red colour when seen in bulk.

4. The various shades of orange and red observed in the individual crystals are due to the admixture, in varying relative proportions, of the above two pigments; and although crystals coloured by the yellow pigment alone are sometimes met with, uroerythrin is never the sole colouring matter of the natural sediments.

5. The minute quantity of iron present in the sediments is not a constituent either of the yellow pigment or of uroerythrin.

6. Other pigments occasionally present in urine, which have a share in the coloration of the crystals in some cases, are the brown products produced by the action of mineral acids, the oxidation products of phenol derivatives, and the pigments of the bile.

7. Urobilin and hæmatoporphyrin take no part in the coloration of the crystals.

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DESCRIPTION OF PLATE III.

Fig. 1.—Crystals of pure, colourless uric acid.

Fig. 2.—Crystals of uric acid deposited from a solution of uroerythrin.

Fig. 3.—Crystals of uric acid deposited from a solution of the yellow pigment of urine (urochrome), in which some colourless urate had been dissolved.

Fig. 4.—Crystals of uric acid deposited from a specimen of urine rich in biliverdin.

Fig. 5.—Crystals of uric acid thrown down by the addition of hydrochloric acid to normal urine.

Fig. 6 —Crystals of uric acid deposited from the dark urine of a patient with carboluria.